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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS :	Perrin et al.		
SERIAL NUMBER :	09/314,698	EXAMINER :	Juliet C. Einsmann
FILING DATE :	May 19, 1999	ART UNIT :	1655
FOR :	Micro-Array Based Subtractive Hybridization		

Assistant Commissioner for Patents
Washington, D.C. 20231

RESPONSE AND AMENDMENT

This paper is in response to the December 10, 2001 Office Action ("Office Action"). Applicants submit herewith a Request for Continuing Examination and a Petition for a Three-Month Extension of Time, along with the appropriate fees. With this extension of time, these documents are due on or before Monday, June 10, 2002. The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Reference No. 21127-501 RCE.

Please amend the application as set forth below and consider the following remarks:

In the Claims:

Please cancel claims 1-10 and 12-26.

Please add the following new claims.

27. (New) A method for identifying a non-redundant nucleic acid in a sample of nucleic acid fragments, the method comprising:

(a) providing a random sample of nucleic acid fragments immobilized on a microarray;

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(b) hybridizing one or more labeled probes corresponding to previously arrayed or sequenced fragments, the sequence of which fragements is known;

(c) identifying at least one immobilized fragment that hybridizes weakly or does not hybridize to the labeled probes;

(d) amplifying a fragment identified in step (d) that does not hybridize to the labeled probes, thereby amplifying a non-redundant nucleic acid fragment in said sample; and

(e) sequencing said amplified non-redundant nucleic acid fragment on said microarray,

thereby identifying a non-redundant nucleic acid.

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28. (New) The method of claim 27, wherein the sample of nucleic acid fragments comprises DNA.

29. (New) The method of claim 27, wherein the DNA is cDNA.

30. (New) The method of claim 27, wherein the DNA is genomic DNA.

31. (New) The method of claim 27, wherein the DNA is a clone from a library.

32. (New) The method of claim 27, wherein the sample of nucleic acid fragments comprises RNA.

33. (New) The method of claim 27, wherein the nucleic acid fragments in said sample are amplified.

34. (New) A method for identifying multiple non-redundant sequences present in low abundance in a random sample of nucleic acid sequences, the method comprising:

(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the random sample of nucleic acids on a solid surface in a microarray format;

(c) hybridizing labeled probes from a DNA source to the immobilized nucleic acid fragments;

(d) identifying at least two immobilized fragments that hybridize weakly or do not hybridize to the labeled probe;

(e) amplifying the at least two immobilized fragments identified in step (d); and

(f) sequencing the at least two fragments amplified in step (e) without isolating said fragments,

thereby identifying multiple non-redundant sequences in low abundance in said random sample.

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35. (New) The method of claim 34, wherein the sample of nucleic acid fragments comprises DNA.

36. (New) The method of claim 34, wherein the DNA is cDNA.

37. (New) The method of claim 34, wherein the DNA is genomic DNA.

38. (New) The method of claim 34, wherein the DNA is a clone from a library.

39. (New) The method of claim 34, wherein the sample of nucleic acid fragments comprises RNA.

40. (New) The method of claim 34, wherein the nucleic acid fragments in said sample are amplified.

41. (New) A method for identifying multiple non-redundant sequences present in low abundance in a random sample of nucleic acid sequences, the method comprising:

(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the random sample of nucleic acids on a solid surface in a microarray format;

(c) hybridizing labeled probes from a DNA source to the immobilized nucleic acid fragments;

(d) identifying at least one immobilized fragment that hybridizes weakly or does not hybridize to the labeled probe;

(e) amplifying the immobilized fragment identified in step (d);

(f) determining the sequence of the fragment amplified in step (d) on said microarray; and

(f) reiterating steps (b) or (c) through (f),

thereby identifying multiple non-redundant sequences in low abundance in said random sample.

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42. (New) The method of claim 41, wherein the sample of nucleic acid fragments comprises DNA.

43. (New) The method of claim 41, wherein the DNA is cDNA.

44. (New) The method of claim 41, wherein the DNA is genomic DNA.

45. (New) The method of claim 41, wherein the DNA is a clone from a library.

46. (New) The method of claim 41, wherein the sample of nucleic acid fragments comprises RNA.

47. (New) The method of claim 41, wherein the nucleic acid fragments in said sample are amplified.

48. (New) A method for identifying multiple nucleic acid fragments corresponding to a whole genome or subregions of interest, the method comprising:

(a) amplifying a random sample of genomic nucleic acid fragments from a whole genome or subregion of interest;

(b) immobilizing the random nucleic acids on a solid surface in a microarray format;

(c) hybridizing labeled probes from a DNA source to the immobilized, microarrayed fragments;

(d) detecting DNA fragments which hybridize to the labeled probe;

(e) determining the identity of the DNA fragment by DNA sequencing of the fragment identified in step (d) on said microarray; and

(f) reiterating steps (b) or (c) through (e),

thereby identifying multiple nucleic acid fragments corresponding to a whole genome or subregions of interest.

49. (New) A method for identifying nucleic acid sequences that are present in different amounts in a first source and a second source, the method comprising:

(a) amplifying and providing a random sample of nucleic acid fragments;

(b) immobilizing the nucleic acid fragments on a coated glass surface in a microarray format;

(c) hybridizing labeled probes from a first source and labeled probes from a second source to the immobilized nucleic acid fragments;

(d) detecting nucleic acid fragments which hybridize to a labeled probe from the first source but which do not hybridize to a labeled probe from the second source; and

(e) sequencing the DNA fragment detected in step (d) on said microarray,

thereby identifying nucleic acid sequences that are present in different amounts in a first source and a second source.

50. (New) The method of claim 49, wherein the random sample of nucleic acid fragments is amplified by PCR or nucleic acid isolation procedures.

51. (New) The method of claim 49, wherein the labeled probes are cDNA, mRNA or genomic sequences.

52. (New) A method for identifying changes in copy number of DNA sequences between different sources of nucleic acids, comprising:

(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the fragments on a coated glass surface in a microarray format;

(c) hybridizing labeled probes from another source to the immobilized fragments;

(d) detecting fragments which show absent, significantly lesser, or significantly greater hybridization to a labeled probe relative to fragments from another source; and

(e) sequencing the DNA fragment detected in step (d) on said microarray, thereby identifying changes in copy number of DNA sequences between different sources of nucleic acids.

53. (New) The method of claim 52, wherein the copy number of DNA sequences is under-represented.

54. (New) The method of claim 52, wherein the copy number of DNA sequences is over-represented.

55. (New) A method for increasing discovery of related DNA sequences comprising:

(a) amplifying a random sample of nucleic acid fragments;

(b) immobilizing the random nucleic acids on a solid surface in a microarray format;

(c) hybridizing labeled probes to the immobilized, microarrayed DNA fragments, particularly at decreased hybridization stringencies;

(d) detecting one or more DNA fragments which hybridize weakly to a labeled probe;

(e) sequencing the one or more DNA fragments detected in step (d) on said microarray; and

(f) comparing DNA sequences obtained to other available DNA sequences to detect sequences which show homology but are not identical to other known sequences.

56. (New) The method of claim 55, wherein the labeled probes are cDNA, mRNA or genomic sequences.

57. (New) The method of claim 55, wherein the labeled probes are pooled labeled probes or single labeled probes.

REMARKS

Upon entry of this amendment, claims 27-57 are pending in the instant application. In this response, claims 1-10 and 12-26 have been cancelled without prejudice or disclaimer, and new claims 27-57 have been added. The claims, as amended herein, are fully supported by the instant specification and the claims as originally filed. Accordingly, no new matter has been added.

35 USC § 103(a)

Kayne et al. in view of Gress et al.

Claims 1-10, 12-14, 21, 25 and 26 have been rejected under 35 USC § 103(a) as being unpatentable over Kayne et al. in view of Gress et al. Since Applicants have cancelled claims 1-10, 12-14, 21, 25 and 26, this rejection as applied to these claims is moot. Applicants believe that new claims 27-48 are non-obvious for the reasons discussed below.

The amended claims now require providing a random sample of nucleic acid fragments of unknown sequence immobilized on a microarray, contacting the microarray with labeled probes, the sequence of which is known, and identifying a fragment in the array to which no probe has hybridized (or to which a probe hybridized only weakly).

In response to Applicants' previous arguments, the Examiner states:

> The fundamental difference between the method of Kayne *et al.* in the instantly claimed method is that in the methods of Kayne *et al.* undefined sequences are in solution and the defined nucleic acid sequences are bound to a solid support. Beyond that difference, the teachings of Kayne *et al.* meet all the limitations of the instant claims as discussed in the rejections in paper number 8. Gress *et al.* clearly provide the teaching and motivation for changing the method of Kayne *et al.* so as to anchor the undefined sequences in the method taught by Kayne *et al.* onto a solid support, as Gress *et al.* specifically teach that methods which employ such a step allow for screening of thousands of clones simultaneously as well as the possibility of automation. (Paragraph spanning pages 8 and 9 of Office action.)

and

> As discussed above, Gress *et al.* is relied upon merely for the suggestion to modify the teaching of Kayne *et al.* so that the undefined sequences are bound to a solid support. (Page 9, lines 12-14 of Office action).

Kayne *et al.* describe making a subtraction library by allowing a library of DNAs of unknown sequences to hybridize to an immobilized collection of DNAs of known sequence, the "driver collection" and recovering the non-hybridizing DNAs (*i.e.* a soluble mixture of unknowns) to yield a subtraction library. Gress *et al.* describe a method of hybridization fingerprinting in which a cDNA library (*i.e.* a mixture of DNAs of unknown sequence) is immobilized and contacted with a second mixture of DNAs of unknown sequence (*i.e.* labeled pooled cDNA from whole animal tissue, *e.g.* liver, ovary, testes, and kidney.)

The Examiner argues that the combination of Kayne *et al.* and Gress *et al.* would have led the ordinary practitioner to use the labeled probes taught by Kayne in solution. Applicants assert that there is no motivation or rationale in either of the cited references to immobilize DNA fragments of unknown sequence and contact the immobilized unknowns with a solution of DNAs of known sequence because both the immobilized and soluble DNAs of Gress are of unknown sequence.

The only discussion of sequence-defined DNAs in the cited art is in Kayne *et al.*, who describe the defined DNAs as immobilized. Kayne *et al.* does not suggest any alternative configuration. Gress fails to discuss DNAs of known sequence at all. Applicants therefore submit that Gress also fails to suggest providing DNAs of a known sequence in solution and hybridizing the known sequence to unknown sequences immobilized on a solid substrate such as a microarray. The Examiner has pointed to no disclosure in either reference, which would lead an ordinary practitioner to alter the teachings of Kayne *et al.* with regard to the "driver collection". Thus, the invention as now claimed is non-obvious over the combination of Kayne *et al.* in view of Gress *et al.*

Claim 27 further requires that the non-redundant nucleic acid fragment is amplified (See step (e)) and sequenced (See step (f)) on the surface of the microarray. Neither Kayne et al. nor Gress et al. teach or suggest the combination of steps (e) and (f) as recited in claim 27. In fact, steps (e) and (f) of the present invention cannot be performed using the method of Kayne et al., since Kayne et al. requires that the non-hybridizing unknown nucleic acid fragments are recovered and isolated from solution prior to sequencing (See Kayne et al., page 2, lines 10-11). Even if one were to substitute the undefined nucleic acids on a surface for the defined nucleic

acids, there would be no way to either identify the non-hybridizing undefined nucleic acids by sequencing without additional steps not required in the present invention. Nor would it be possible to identify the non-hybridizing defined nucleic acids without recovery and isolation steps not required by the present invention. Therefore, Kayne cannot guide the ordinary practitioner in how to amplify a non-hybridizing nucleic acid fragment and then sequence this amplified fragment on the microarray.

Gress does not cure this deficiency, since it does not recite the steps of on-array amplification and sequencing. Gress teaches that cDNA inserts are amplified "from a single colony of the particular cDNA clone". (See Gress et al., p. 611 column 1). Further, nucleic acid sequencing is performed on isolated plasmid DNA. (See Gress et al., p. 611 column 1).

The Examiner states that "Gress et al. clearly provide the teaching and motivation for changing the method of Kayne et al. so as to anchor the undefined sequences in the method taught by Kayne et al. onto a solid support...". (See Office Action, page 9). However, as discussed above, Kayne et al. teach that the non-hybridizing sequences are in solution and must be recovered and isolated prior to identification by nucleic acid sequencing. Therefore, Kayne provides no guidance on how to identify the non-hybridizing nucleic acid sequences on the array. Thus, even if one skilled in the art, using Kayne et al. in view of Gress et al., was to "have spotted the library of random nucleic acids on the microarray in order to have provided an improved method for isolating and identifying non-redundant nucleic acids," (See Office Action, pages 3-4), one would not have been able to identify those non-hybridizing sequences. Therefore, new claim 27 is not obvious in view of Kayne et al. and Gress et al.

New claims 28-33 depend on claim 27, and are therefore non-obvious as well. New independent claims 34 and 41 also recite the limitation that the non-redundant nucleic acid fragment is amplified and sequenced on the surface of the microarray, and new claims 35-40 and 42-47 depend on either claims 34 or 41. Therefore, these claims are also non-obvious in view of the arguments discussed above. Step (e) of new claim 48 requires that the identity of the DNA fragment is determined by sequencing the fragment on the microarray surface. As stated above, neither Kayne et al., Gress et al., or a combination of the two references disclose or suggest a method of sequencing the identified DNA fragment on the microarray surface. Therefore, claim 48 is non-obvious in view of these references.

Pinkel et al. in view of Schena et al.

Claims 15-17 and 22-24 have been rejected under 35 USC § 103(a) as being unpatentable over Pinkel et al. in view of Schena et al. Since Applicants have cancelled claims 15-17 and 22-24, this rejection as applied to these claims is moot. Applicants believe that this rejection does not apply to new claims 49-57 for the reasons discussed below.

The Examiner states that "Pinkel et al. disclose a method for enrichment and/or isolation of DNA sequences that are unique to a population", and further states that Schena et al. teach "methods for monitoring gene expression patterns using a microarray spotted onto a class microscope slide." (See Office Action at pages 5-6). The Examiner concludes that "[i]t would have been prima facie obvious to . . . have spotted the random nucleic acid fragments taught by Pinkel et al. onto a glass slide as taught by Schena et al." (See Office Action at page 6).

Applicants have added new claims 49-57 and assert that these claims are non-obvious in view of Pinkel et al. and Schena et al.

New claim 49 reads in part:

"(d) detecting nucleic acid fragments which hybridize to a labeled probe from the first source but which do not hybridize to a labeled probe from the second source; and

(e) sequencing the DNA fragment detected in step (d) on said microarray,

thereby identifying nucleic acid sequences that are present in different amounts in a first source and a second source."

Step (e) of new claim 49 requires that the DNA fragment detected in step (d) is sequenced on the microarray surface. Neither Pinkel et al. nor Schena et al. teach or suggest that the detected nucleic acid fragment can be sequenced on the microarray surface. Pinkel et al. discuss using FISH (fluorescence *in situ* hybridization) to genetically or physically map the clones used as binding targets (See Pinkel et al. column 17, lines 46-50). However, while FISH provides a chromosomal localization, this localization only defines a region comprising approximately 2% of the chromosome length (See Lichter et al. Science, 247:66 (1990), cited in Pinkel et al.) and thus does not provide a nucleic acid sequence for the nucleic acid fragment. Schena et al. teach that the cDNAs deposited on the glass microscope slides were sequenced prior to spotting (See Schena et al., page 467, columns 2-3). Therefore, neither Pinkel et al. or Schena et al. either

teach or suggest the limitations of new claim 49.

New claims 50-51 depend on claim 49, and are therefore non-obvious as well. New independent claim 52 also recites the limitation that the detected nucleic acid fragments are sequenced on the surface of the microarray, and new claims 53-54 depend on claim 52. Therefore, these claims are also non-obvious in view of the arguments discussed above.

Pinkel et al. in view of Schena et al. and in view of Maslyn et al.

Claims 18-20 have been rejected under 35 USC § 103(a) as being unpatentable over Pinkel et al. in view of Schena et al. in view of Maslyn et al. Since Applicants have cancelled claims 18-20, this rejection as applied to these claims is moot. Applicants believe that this rejection does not apply to new claims 55–57 for the reasons discussed below.

The Examiner states that Maslyn et al. teach that "a cluster is a group of clones related to one another by sequence homology...". The Examiner concludes that "[i]t would have been prima facie obvious to . . . have included the comparison step of Maslyn et al. in the method taught by Pinkel et al." (See Office Action at page 8).

Applicants have distinguished the present invention from Pinkel et al. and Schena et al in part based on the lack of teaching or suggestion of these two references to sequence a detected nucleic acid on a microarray surface. Applicants assert that Maslyn et al. adds nothing to overcome the deficiencies of Pinkel et al. and Schena et al. Maslyn et al. relates generally to relational databases for storing and retrieving biological information (See Maslyn et al., abstract). Maslyn et al. do not teach or suggest the sequencing of a detected nucleic acid on a microarray surface. Applicants therefore contend that the present invention is non-obvious in view of Pinkel et al., Schena et al., and Maslyn et al., and that the 35 USC § 103(a) rejection can be withdrawn.

The Commissioner is hereby authorized to charge any additional fees that may be due, or credit any overpayment of same, to Deposit Account No. 50-0311, Attorney Reference No. 21127-501 RCE. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

Kevin M. Coughlin, Reg. No. 48,593

Ivor R. Elrifi, Reg. No. 39,529
Ingrid Beattie, Reg. No. 42,306
Attorneys for Applicants
c/o MINTZ, LEVIN
One Financial Center
Boston, Massachusetts 02111
Tel: (617) 542-6000
Fax: (617) 542-2241

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